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Biological Control of Tobacco Cutworm, *Spodoptera litura* Fabricius with Entomopathogenic Nematodes

Sun Ho Park[1,2]*, Yeon Su Yu[1,2], Jae Sung Park[1,2], Ho Yul Choo[3], Soon Do Bae[4], and Min Hee Nam[4]

[1] Faculty of Engineering, Keimyung University, Daegu 704-701, Korea
[2] Biotechnology Research Institute, Advanced Biocontrol System Co., Ltd., Daegu 704-701, Korea
[3] Department of Agricultural Biology, Gyeongsang National University, Chinju 660-701, Korea
[4] National Yeongnam Agricultural Experiment Station, RDA, Milyang 627-130, Korea

Abstract The efficacies of several entomopathogenic nematodes of *Steinernema* and *Heterorhabditis* spp. were examined against tobacco cutworm, *Spodoptera litura* Fabricius. *H. bacteriophora* HY showed 100% mortality after 20 h against 2nd instar of tobacco cutworm. In the case of 3-4th instar, *S. carpocapsae* PC, *H. bacteriophora* HY and *S. monticola* CR showed 100% mortality after 47 h. In the case of 5-6th instar, *S. carpocapsae* PC proved more effective than the others. Generally, the number of nematodes harvested increased as their size decreased. Also, the highest number of nematodes was obtained in the 5-6th instar of *S. litura* by *H. bacteriophora* HY, showing about 1.3×10^6 nematodes per larva. *In vitro* cultured *S. carpocapsae* PC showed 100% mortality after 73 h against 5-6th instar tobacco cutworm, indicating that nematodes produced *in vitro* can be potentially used for the biological control of *S. litura* instead of nematodes *in vivo*.

Keywords: biological control, *Steinernema*, *Heterorhabditis*, entomopathogenic nematode, tobacco cutworm, *Spodoptera litura*

INTRODUCTION

Entomopathogenic nematodes of the Steinernematidae [1] and Heterorhabditidae [2] families, which belong to the Rhabditida order, are useful biopesticides interacting with symbiotic bacteria *Xenorhabdus* spp. [3,4]. Entomopathogenic nematodes are up-and-coming biological control agents for the next generation due to their various habitats, wide range of insect hosts, excellent ability at searching hosts, easiness of mass culture, and ability to mingle with chemical pesticides [5-9].

Spodoptera litura Fabricius is a subtropical insect pest that is found largely in Korea, Japan and China and damages broad leaf plants like leguminous, cruciferous, and other economically important crops [10,11]. As the number of equipped green house increases, it is found throughout the year and sometimes occur in very large numbers under certain environmental conditions [12]. Larvae of 4-5th instar have high tolerance to chemical pesticides [13]. Microbial control of the tobacco cutworm using *S. litura* nuclear polyhedrosis virus has been also reported [14].

In this paper, we examined the efficacies of Steinernematidae, *S. carpocapsae*, *S. glaseri*, *S. monticola*, *Steinernema longicaudum* and *Heterorhabditis bacteriophora* of Heterorhabditidae against *Spodoptera litura* Fabricius: and investigated not only their effectiveness but also their effect on the mortality of larvae in the developmental stages, their multiplication, and the pathogenic difference between nematodes cultured *in vitro* and *in vivo* according to their concentrations.

* Corresponding author
Tel: +82-53-580-5457 Fax: +82-53-633-4929
e-mail: park@kmu.ac.kr

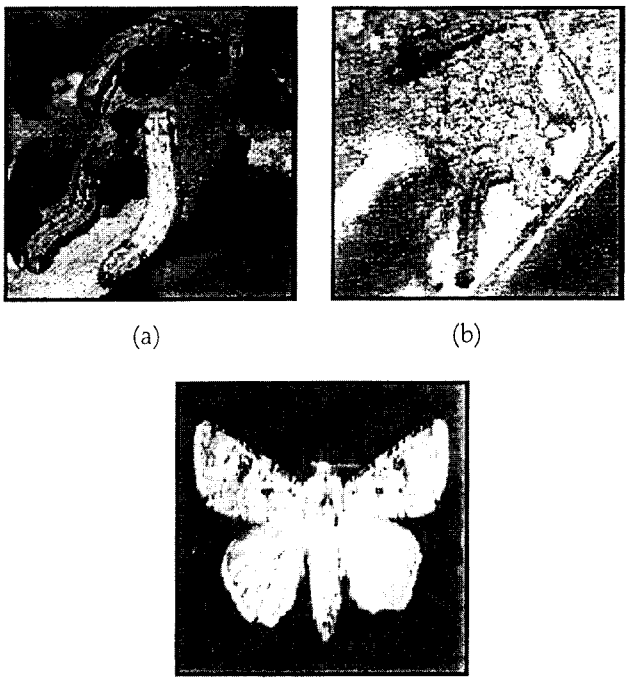
MATERIALS AND METHODS

Nematodes

Seven entomopathogenic nematode species in the Steinernematidae and Heterorhabditidae families were maintained continuously using *Galleria mellonella* larva and used in this study [15]. Six nematode species were isolated in Korea (*S. glaseri* DR form Dongrae, *S. glaseri* MK from Munkyeong, *S. carpocapsae* PC from Pocheon, *S. monticola* CR from Chirisan, *S. longicaudum* GJ from Gongju and *H. bacteriophora* HY from Hamyang) and 1 species of *S. glaseri* NC was isolated from North Carolina in the USA.

Control of Entomopathogenic Nematodes against the Larvae of the Tobacco Cutworm

Larvae of the tobacco cutworm, *Spodoptera litura* Fabricius used in this study were obtained from the Yeongnam Agriculture Experiment Station, RDA (Fig. 1). Filter papers (ϕ 50 mm, Whatman No. 2), spread suspension (0.3 mL, approximately 300 nematodes per



(a) (b) (c)

Fig. 1. Life cycle of tobacco cutworm, *Spodoptera litura* Fabricius. (a) Damaged leaf, (b) Larva, (c) Adult.

larva) and 7 species of *in vivo* cultured nematodes were placed in petridishes(50 mm diameter). Classified larvae of 2nd, 3rd, 4th and 5-6th instar were used. The larva were fed with kale leaves. For each species of nematode, 10 larvae were examined, mortality were investigated in an incubator for 73 h at 28°C. As a control, the mortality of the larvae were investigated over filter papers, without nematodes, in identical petridishes but with only 0.3 mL of distilled water spread over the filter papers.

Multiplication of Entomopathogenic Nematodes

Filter paper (ϕ 50 mm, Whatman No. 2) with suspension containing (0.3 mL, approximately 300 nematodes per larva) several species of infective juvenile nematodes were placed in petridishes and inoculated tobacco cutworms introduced according to the developmental stages of the larvae. After 7 days, the larva were anatomized and live nematodes separated using the water trap method [16], their development was investigated using a light microscope.

Pathogenicity of Nematodes Cultured *in vitro* and *in vivo*

In vivo and *in vitro* cultured nematodes of *S. carpocapsae* were prepared using the methods previously reported [7,8]. Filter paper (ϕ 50 mm, Whatman No. 2) with suspension (0.3 mL, approximately 300 nematodes per larva) were placed in petridishes and tobacco cut

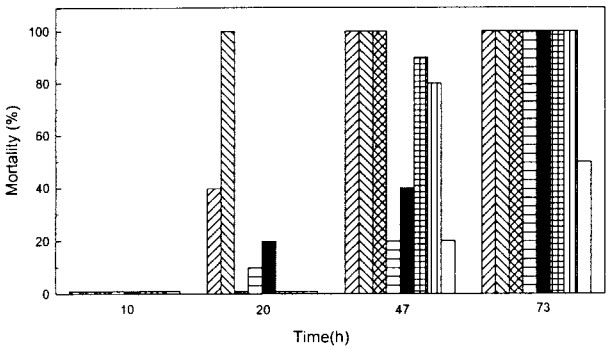


Fig. 2. Mortalities of 2nd instar of *S. litura* by various nematodes. (▨) *S. carpocapsae* PC, (▧) *H. bacteriophora* HY, (▩) *S. monticola* CR, (▤) *S. longicaudum* GJ, (■) *S. glaseri* MK, (▦) *S. glaseri* NC, (▥) *S. glaseri* DR, (□) control.

worms introduced according to the developmental stages of the larvae. For each species of nematodes, 10 larvae were used to investigate mortality in an incubator for 73 h at 28°C. We also determined the mortality of larvae over filter papers without nematodes in a petridish and with only 0.3 mL of distilled water spread over filter papers as a control. *S. carpocapsae* nematodes produced *in vivo* and *in vitro* were used at a concentration of approximately 50, 150, 300 and 600 nematodes per larva of 5-6th instar tobacco cutworm.

RESULTS AND DISCUSSION

Control Efficacies of Entomopathogenic Nematodes against Tobacco Cutworm in Each of Its Developmental Stages

The effect of *S. carpocapsae* PC, *H. bacteriophora* HY, *S. longicaudum* GJ and *S. glaseri* MK against 2nd instar larvae of tobacco cutworm was expressed 20 h after inoculation as shown in Fig. 2, and the 7 species of nematodes produced 100% mortality in the 2nd larvae of tobacco cutworm in 73 h. Particularly, *H. bacteriophora* HY showed 100% mortality in 20 h while *S. longicaudum* GJ and *S. glaseri* MK showed their efficacies comparatively late.

For the 3rd instar larvae of tobacco cutworm, as shown in Fig. 3, *H. bacteriophora* HY, *S. longicaudum* GJ, *S. glaseri* MK and *S. glaseri* NC started working 20 h after inoculation. *S. carpocapsae* PC, *H. bacteriophora* HY and *S. monticola* CR all showed 100% mortality in 47 h, and after 73 h all of the nematodes except *S. glaseri* NC (90%) produced 100% mortality. Though the expressions of *S. glaseri* DR and *S. glaseri* NC were comparatively later than the others and were comparable with the case of the 2nd instar larvae.

Unlike 2nd and 3rd instar of tobacco cutworm, nematodes showed about 0% mortality in 20 h against 4th instar as shown in Fig. 4. 47 h after inoculation, *S. carpocapsae* PC and *H. bacteriophora* HY showed 100%

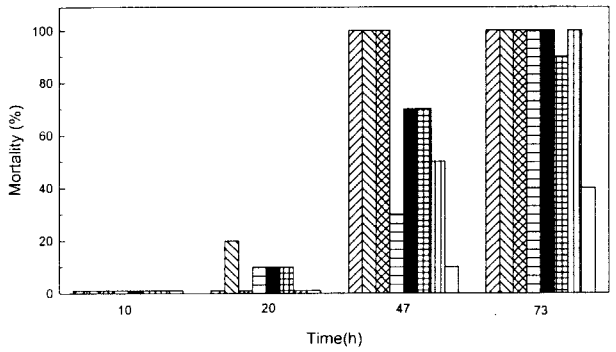


Fig. 3. Mortalities of 3rd instar of *S. litura* by various Nematodes. (▨) *S. carpocapsae* PC, (▧) *H. bacteriophora* HY, (▩) *S. monticola* CR, (▤) *S. longicaudum* GJ, (■) *S. glaseri* MK, (▦) *S. glaseri* NC, (▥) *S. glaseri* DR, (□) control.

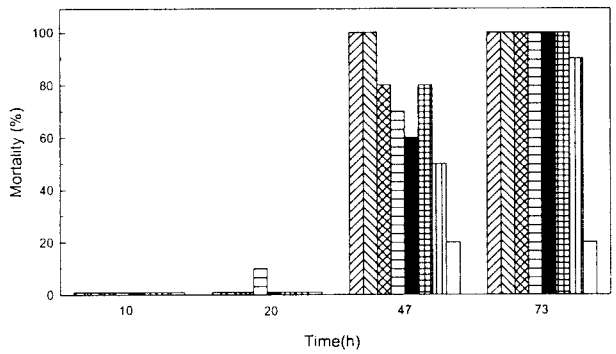


Fig. 4. Mortalities of 4th instar of *S. litura* by various Nematodes. (▨) *S. carpocapsae* PC, (▧) *H. bacteriophora* HY, (▩) *S. monticola* CR, (▤) *S. longicaudum* GJ, (■) *S. glaseri* MK, (▦) *S. glaseri* NC, (▥) *S. glaseri* DR, (□) control.

mortality and 50-80% pathogenicity in the other 5 species. After 73 h, all but *S. glaseri* DR (90%) showed 100% mortality.

For 5-6th instar tobacco cutworms, *S. longicaudum* GJ, *S. glaseri* NC and *S. glaseri* DR showed approximately 40% pathogenicity after 20 h, as shown in Fig. 5. After 47 h, all of the 6 species of nematodes except *S. glaseri* MK (40%) showed more than 70% mortality. After 73 h, all but *S. glaseri* MK (70%) showed 90% or more mortality. *S. glaseri* DR (890 ± 138 μm), *S. glaseri* NC (980 ± 123 μm) and *S. longicaudum* GJ (980 ± 123 μm), which were comparatively potent in the early period, were longer than *S. glaseri* MK (590 ± 30 μm), *H. bacteriophora* HY (570 ± 46 μm) and *S. carpocapsae* PC (590 ± 30 μm) at the infective juvenile stage. Nematodes penetrated the larvae through the mouth, anus, stoma and surface of the skin: as the size of larvae increased, larger nematodes could probably penetrate more easily. Moreover, it was confirmed that small nematodes could penetrate easily into 2nd and 3rd instar animals and were rapidly effective but they produced lower mortality during the early period in case of 4-6th instar.

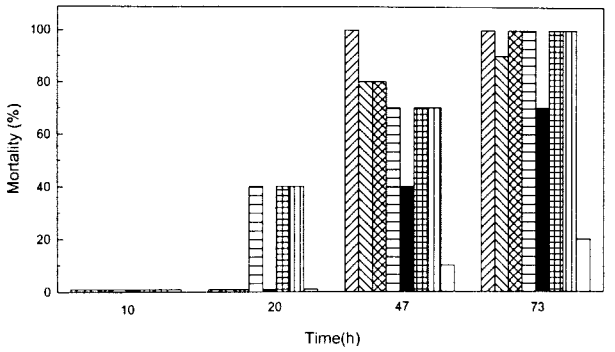


Fig. 5. Mortalities of 5-6th instar of *S. litura* by various Nematodes. (▨) *S. carpocapsae* PC, (▧) *H. bacteriophora* HY, (▩) *S. monticola* CR, (▤) *S. longicaudum* GJ, (■) *S. glaseri* MK, (▦) *S. glaseri* NC, (▥) *S. glaseri* DR, (□) control.

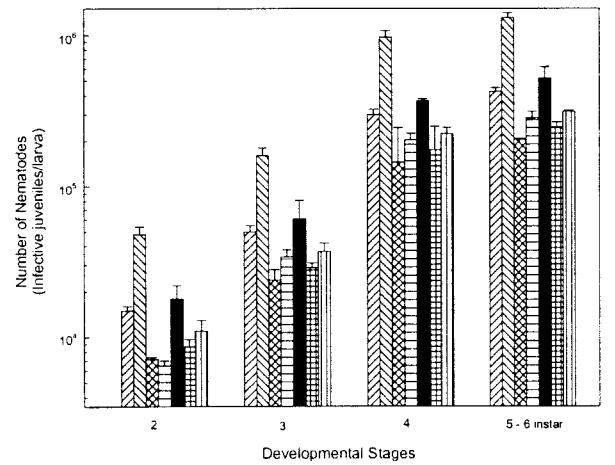


Fig. 6. The number of various nematodes harvested in the different developmental stages of *S. litura*. (▨) *S. carpocapsae* PC, (▧) *H. bacteriophora* HY, (▩) *S. monticola* CR, (▤) *S. longicaudum* GJ, (■) *S. glaseri* MK, (▦) *S. glaseri* NC, (▥) *S. glaseri* DR.

Multiplication of Entomopathogenic Nematodes

The multiplication of nematodes against the larvae of tobacco cutworm was examined by comparing the number of nematodes, as shown in Fig. 6. 300 nematodes were inoculated per larva in its each developmental stages, and the number of nematodes harvested after inoculation was compared with this. From 2nd larvae, we could obtained more than 6×10^3 nematodes of all species per larva. Approximately 2×10^4 - 2×10^5 nematodes per larva from the 3rd instar, 1×10^5 - 1×10^6 nematodes per larva from the 4th instar and 2×10^5 - 2×10^6 nematodes per larva from the 5-6th instar. The number of nematodes harvested was directly proportional to the weight of the larvae, which varied as follows: 2nd instar weigh 0.030 ± 0.005 g, 3rd instar weigh 0.1 ± 0.055 g, 4th instar weigh 0.2 ± 0.022 g, 5-6th instar weigh 0.899 ± 0.003 g. The highest number of

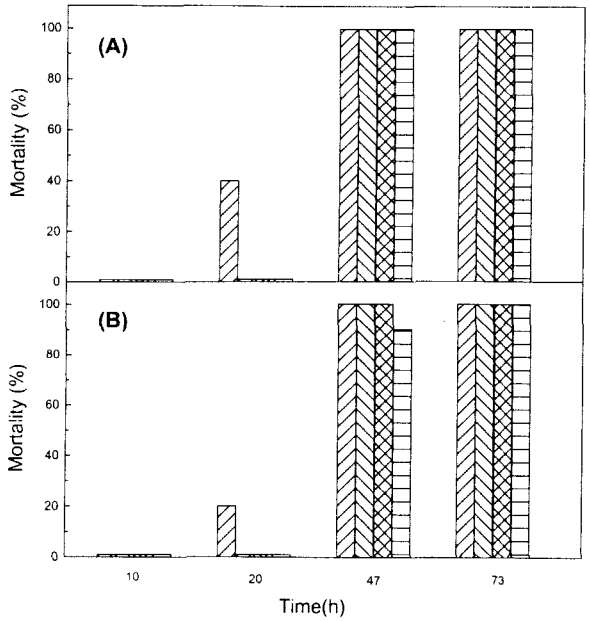


Fig. 7. Mortalities of different developmental stages of *S. litura* by *S. carpocapsae* cultured *in vivo* and *in vitro*. (A) *in vivo* cultured nematodes, (B) *in vitro* cultured nematodes. (▨) 2nd instar, (▧) 3rd instar, (▩) 4th instar, (▤) 5-6th instar.

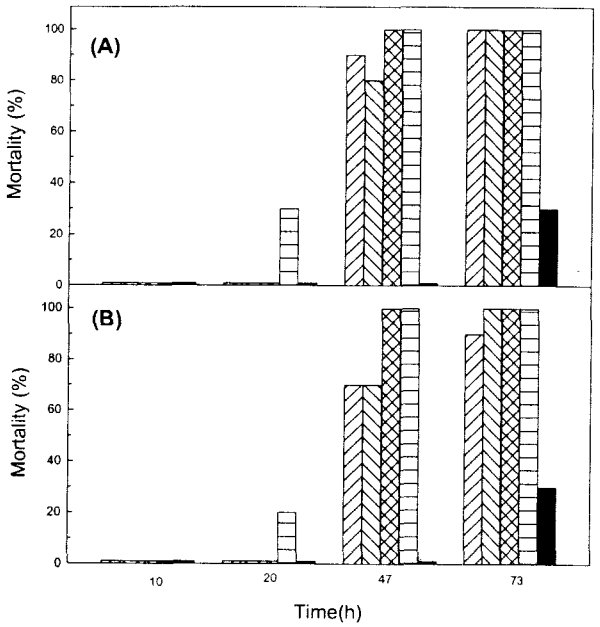


Fig. 8. Mortalities of 5-6th instar of *S. litura* by various concentrations of infected nematode concentration per larva. (A) *in vivo* cultured nematodes, (B) *in vitro* cultured nematodes. (▨) 50, (▧) 150, (▩) 300, (▤) 600, (■) control.

nematodes harvested was achieved by two species, *S. carpocapsae* PC and *H. bacteriophora* HY. In case of *S. carpocapsae* PC, the number of nematodes as follows: 1.5×10^4 nematodes from 2nd instar, 5.0×10^4 from 3rd instar, 3.0×10^5 from 4th instar and 4.3×10^5 from 5-6th instar and *H. bacteriophora* HY varied at a rate of 1 : 3.3 : 20.2 : 27 – 4.8×10^4 nematodes from 2nd instar, 1.61×10^5 from 3rd instar, 9.7×10^5 from 4th instar and 1.3×10^6 from 5-6th instar. The number of *S. carpocapsae* PC in the body of larvae increased to 500 nematodes per 0.01 g.

Pathogenicity of Nematodes Cultured *in vitro* and *in vivo*

Fig. 7 shows the pathogenicity differences between the *in vitro* and *in vivo* cultured nematodes of *S. carpocapsae* PC. 300 nematodes were inoculated per larva in its each developmental stage. After 20 h, 2nd instar larvae, which were comparatively small, showed high mortality. After 47 h, both *in vivo* cultured and *in vitro* cultured nematodes all showed 90-100% mortality.

Fig. 8 shows pathogenicity differences according to concentration. The comparison was done using *S. carpocapsae* PC cultured *in vivo* and *in vitro*. The nematodes were inoculated against the 5-6th instar larvae at 50, 150, 300 and 600 nematodes per larva. After 20 h, both *in vivo* and *in vitro* cultured nematodes were more effective when they were inoculated at 600 rather than at 50-300. After 73 h, *in vivo* and *in vitro* cultured nematodes all showed more than 90% mortality.

CONCLUSION

To investigate the biological control of tobacco cutworm, *Spodoptera litura* Fabricius using entomopathogenic nematodes, we examined mortality in each developmental stage of the insect larva, the multiplication of nematodes, and the pathogenicity difference between *in vivo* and *in vitro* cultured nematodes at different concentration. Tobacco cutworms are often found in packages of beans or perilla. A throng of 1st and 2nd instar larvae damages the body of leaves and after the 3rd instar, serious damage is done to the leaves. When they get older they hide under the surface of the ground in the daytime and move out at night. They stay 1-2 cm under the surface until they pupate. Older larvae have a strong tolerance to chemical pesticides and there is no specific control method against them. Entomopathogenic nematodes can search more than 20 cm into the ground and so they are effective even against the older larvae and chrysalises.

This study concerns the biological control of the tobacco cutworm using several entomopathogenic nematodes: *Steinernema carpocapsae, S. glaseri, S. monticola, S. longicaudum* and *Heterorhabditis bacteriophora*. *H. bacteriophora* HY was more effective against the 2-3rd instar larvae while *S. carpocapsae* PC was more effective on 4-6th instar larvae. Among the nematodes examined, the comparatively small species were more multiplicative than the large species: *H. bacteriophora* HY was the most multiplicative showing 1.3×10^6 nematodes harvested per larva. There was no observable difference between *in vivo* and *in vitro* cultured *S.*

carpocapsae PC. It is possible to control tobacco cutworm with more than 50 nematodes per larva and as this concentration increases mortality in early period also increases.

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